was to analyze the impact of CsA levels in the development of grade 2-4 aGVHD in the setting of allo-RIC in the first four weeks after transplantation.

Patients and Methods: We included 156 consecutive patients [64 (41%) women], median age 52 (17-69) years, who underwent HLA-identical sibling allo-RIC at a single institution. RIC included fludarabine 150 mg/m2 plus busulfan 10 mg/kg (for myeloid malignancies n = 53) or melphalan 70-140 mg/m2 (lymphoid malignancies n = 103). GVHD prophylaxis was based on CsA plus methotrexate (MTX) (n = 121, 78%) or mychopenolate mofetil (MMF) (n = 35, 22%). CsA levels were measured at least twice weekly during the first four weeks (or until discharge) and the dose was adjusted to maintain blood levels between 200 and 300 ng/ml.

Results: As the use of MTX vs MMF did not impact on the incidence of grades 2-4 aGVHD patients were analyzed together. The median blood concentrations of CsA at 1st, 2nd, 3rd and 4th weeks after allo-SCT were 134 (95 CI:10-183), 219 (95 CI: 54-261), 253 (95 CI: 53-314) and 224 ng/ml (95 CI:30-411) respectively. The number of patients who were in the optimal range in the 1st, 2nd, 3rd and 4th weeks after allo-RIC were 34/150 (22%), 92/154 (59%), 86/148 (58%) and 53/123 (56%). Sixty six patients developed grade 2-4 aGVHD for a cumulative incidence of 42% (95% CI 35-51%) at a median of 38 (range:18-138) days after allo-SCT. In univariate analysis the variables associated with a higher incidence of 2-4 aGVHD were: male sex ($p = 0.016$), female to male donor-recipient sex combination ($p = 0.05$), and median CsA levels in the second ($p = 0.02$) and third ($p = 0.02$) weeks. In multivariate analysis, the only significant variables associated with higher 2-4 aGVHD were female to male donor-recipient sex combination (HR 2; $p = 0.01$) and the median CsA levels in the third week (HR 0.097, $p = 0.039$).

Conclusion: The levels of CsA in the immediate post-transplant period were suboptimal in almost 50% of patients. Low levels of CsA were associated with higher incidence of grade 2-4 aGVHD. A more stringent monitoring and modification of CsA in the early phase post-Allo-RIC may be helpful to prevent aGVHD.

396

ANALYSIS OF THE FLT3-ITD AND NPM1 MUTATIONS IN AML PATIENTS WITH INTERMEDIATE RISK RECEIVING ALLOGENEIC STEM-CELL TRANSPLANTATION

Ogasawara, M., Yamakawa, T., Katsura, Y., Shima, K., Matsukawa, T., Kanaya, M., Minauchi, K., Nakata, M., Ota, S., Imai, K., Hirano, T., Kobayashi, N., Kiyama, Y. Sapporo Hokuyu Hospital, Sapporo, Japan

Background: Chromosomal abnormality is the most important prognostic factor for AML patients. Recently, prognosis of cytogenetically normal AML patients has been reported to be affected by the presence of fms-like tyrosine kinase 3 gene internal tandem duplication (FLT3-ITD) and nucleophosmin 1 gene (NPM1) mutations. In the present study, we analyzed retrospectively the FLT3-ITD and NPM1 mutations in AML patients with cytogenetically intermediate risk who received allo-SCT and we evaluated the effect of the mutations on the outcome of allo-SCT.

Patients and Methods: 23 patients (11 males and 12 females) with a median age of 46 years (range: 27-65) receiving allo-SCT between 2005 and 2009 whose BM samples were available were enrolled in this study. Allo-SCT consisted of 10 matched siblings, 8 matched unrelated donors and 5 unrelated cord blood. GVHD prophylaxis included CsA/ short term MTX (10) or FK/short term MTX (13). Genomic DNA was extracted from PBMC and amplified by PCR using specific primers. Analysis of FLT3-ITD or NPM1 exon 12 mutations was carried out by either electrophoresis or direct DNA sequencing.

Results: FLT3-ITD mutation was found in 5 patients (21.7%). FLT3-ITD mutation occurred only in cytogenetically normal patients (positivity: 38.5%). On the other hand, NPM1 mutation was found in 6 patients (26.1%). Acute GVHD above grade II was found in 4 patients (57.1%) with FLT3-ITD mutation while only 3 patients (20.0%) without FLT3-ITD mutation manifested the complication. Frequency of acute GVHD above grade II was 33% and 41% in NPM1 mutation positive and negative patients, respectively. All the patients with FLT3-ITD mutation manifested relapse of the disease following allo-SCT while relapse occurred in 5 patients (33.3%) without FLT3-ITD mutation. However, there was no marked difference in relapse rate between patients with or without NPM1 mutation (33% vs. 41%). Median period of overall survival was 0.184 and 1.619 years in FLT3-ITD mutation positive and negative patients, respectively.

Conclusions: It was demonstrated that the rate of both relapse and acute GVHD was significantly higher in AML patients with FLT3-ITD mutation compared to FLT3-ITD mutation negative patients. NPM1 mutation exerted minimal effect on the incidence of acute GVHD and relapse of the disease. These results suggested that allo-SCT patients with cytogenetically intermediate risk can be stratified to poor prognosis group if FLT3-ITD mutation is identified.

397

PLASMA CYTOKINE PROFILES AT DAY ZERO: MYELOABLATIVE CONDITIONING EXHIBITS A MORE INFLAMMATORY PROFILE THEN REDUCED INTENSITY CONDITIONING IN PEDIATRIC PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

Grimley, M.[1], Rehman, N.[1], Mellor Heinke, S.[1], Villanueva, J.[1], Marsh, R.[1], Bleesing, J.[1], Mehta, P.[1], Jordan, M.[1], Kim, M.-O.[2], Li, D.[2], Davies, S.[1], Filopovich, A.[1] [1] Cincinnatio Children's Hospital Medical Center, Cincinnati, OH; [2] Cincinnati Children's Hospital Medical Center, Cincinnati, OH

While myeloablative conditioning (MAC) has been the conventional preparative regimen for allogeneic stem cell transplant, reduced intensity conditioning (RIC) has increasingly been used, especially in non-malignant conditions. MAC has been associated with a cytokine storm that may contribute to graft versus host disease (GVHD) while RIC has shown lower tissue damage which may lead to a lower release of inflammatory cytokines. We hypothesized that patients receiving MAC would express a more inflammatory subset of plasma cytokines on Day Zero compared to patients receiving RIC.

METHODS: We prospectively collected samples on 52 consecutive consented patients who underwent allogeneic transplantation at Cincinnati Children's Hospital Medical Center between December 2007 and October 2008. Blood samples were collected at Day 0. Patient Characteristics are in Table 1.

Table 1.

	RIC	MAC
Number of Patients	23	29
Patient Age -median (range) years	6.7 (0.6-17.9)	8.1 (0.8-19.4)
Patient Gender	13 males/10 females	20 males/9 females
Diagnosis	23 non-malignant	12 malignant/17 non-malignant

A Bio-Plex Pro Assay was used to measure plasma levels of GM-CSF, G-CSF, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, IFN-γ, MCP-1, MIP-1b, TNF-α, IL-18, and MIF. Plasma concentrations of sTNF-R1 were measured by ELISA. Soluble IL-2 Receptor alpha (sIL2Rα) levels were measured using the Immulite platform.

RESULTS: Wilcoxon rank sum test was used to compare the plasma cytokine levels between the RIC and MAC groups. IL-6, G-CSF, sIL-2Rα, IL-17 and IL-7 plasma levels were found to be different in the two groups ($p \leq 0.05$). We additionally analyzed 2 groups within the RIC cohort-10 patients received distal alemtuzumab (between Days -22 and -13 pretransplant) and 12 patients received proximal alemtuzumab (between Days -12 and -8 pretransplant). In this analysis, patients who received distal alemtuzumab have higher levels of the tested cytokines including IL-1b, IL-6, IL-8, IFN-γ, MIF and TNF-α ($p \leq 0.05$).

DISCUSSION: A pro-inflammatory cytokine profile (increased IL-6, G-CSF and sIL-2Rα) is seen in MAC patients when compared to RIC patients who have increased levels of differentiation (IL-17) and growth (IL-7) cytokines. The timing of alemtuzumab prior to transplant affects the cytokine profile on Day 0. Patients receiving distal alemtuzumab have higher levels of the pro-inflammatory cytokines